PROJECT #21 19 XII 72

Fixation of Egg Blastoderm [fertile New Hampshire Eggs]

EMC - 21A - Fresh Eggs

EMC - 21B - Begin storage on 5 XII 72

EMC - 21C - Begin storage on 21 XI 72

Controls of temperature and time were not adequate because of technical problems in fixation procedure.
Glutaraldehyde fixation followed by Osmium - both in $P0_4$ buffer

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Procedure for fixation of egg blastoderm 19 I 73 JDH

1. apply a teflon ring on top of albumin layer surrounding the blastoderm
2. fill the ring with 3% glut. in 0.1M $P0_4$ buffer and drip the fixative solution around the outside of the ring.
3. allow the fixative to remain in the ring for 1 hour at room temperature before attempting to remove the blastoderm.Add fresh fixative to the ring and drip around the ring a few times during the hour.
4. remove the ringand with a Justrite #12 curved blade, slowly make a drawing cut surrounding the blastoderm - about 1/4 in. outside the blastoderm.
5. inject (slowly) fixative under the blastoderm, about 2cc and allow to remain for 5min.
6. with a stainless stell scoopula,slowly insert under the blastoderm and lift free from the yoke.
7. transfer the blastoderm to fresh fixative in a 35x10 petri dish and leave for 1 hour.
8. rinse 3 times in 0.1M $P0_4$ buffer for a total of 1 hour.
9. fix in 1% Osmium in 0.1M P04 buffer for 1 hour at room temperature.
10. dehydration in acetone series of 10 min. ea.
    10%, 30%, 50%, 70% , 100%
11. epon embedding - epon 1:1 acetone open overnite to evaporate
    epon 100% 2days at room temperature
    epon 80C -

Remarks:

To recognize the area of the blastoderm more accurately, implant small hairs into albumin around the outside of the blastoderm.

At step #4 the albumin and some of the yoke under the blastoderm should be firm.

Step #5 may be left out to remove more of the yoke from under the blastoderm.

Osmium does not appear to penetrate even small amounts of the yoke on the back of the blastoderm, so most of the yoke must be removed or the blastoderm cut into very small pieces.

When cutting the blastoderm, cut through the albumin side first.

EMC - 21D - fresh eggs